Short Communication

Shortest Known Prion Protein Allele in Highly BSE-Susceptible Lemurs

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We describe the shortest prion protein allele known to date. Surprisingly, it is found as a polymorphism exactly in a species (prosimian lemurs) which seems highly susceptible to oral infection with BSE-derived prions. The truncation of the prion protein we found raises several questions. First, is the truncated octarepeat structure we describe, consisting of two octarepeats, still functional in copper binding? A second question is whether this truncation is related to the remarkable oral infectibility of lemurs with BSE-derived prions. And finally, one could argue that this genotype alone might favour development of a prion disease, even in the absence of exogenous infection.
Key words: BSE / Lemur / Octarepeats / Prion / Prosimians / PrP allele.

Prion diseases such as kuru, bovine spongiform encephalopathy (BSE) and the new variant of Creutzfeldt-Jakob disease (nvCJD) represent prion disorders acquired by exogenous infection (Prusiner, 1997). Non-human primates have been extensively used for the study of prion infectivity until transgenic mouse models became available (Brown *et al.*, 1994). Nevertheless, non-human primate models are considered extremely valuable for studying prion disease pathogenesis because they represent the experimental model closest related to the situation in human beings. Recently, both natural and experimental oral infection of lemur monkeys by BSE-derived prions was reported (Bons *et al.*, 1999). The pathologic prion protein (PrP^{Sc}) isoform could be detected in affected lemurs in lymphoid tissues and that of the peripheral and the central nervous system prior to the clinical onset of prion disease symptoms. Therefore, these prosimian monkeys might represent a very important experimental model for the study of prion pathogenesis and of tissue distribution of prion infectivity.

The knowledge of prion protein (PrP) primary structures has provided important insights into various putative structural and functional aspects of PrP and into species barrier mechanisms (Schätzl *et al.*, 1995; Wopfner *et al.*, 1999). Although the PrPs of more than 25 non-human primate species have been analyzed (Schätzl *et al.*, 1995), no data for prion protein genes of prosimian species were available. Using PrP gene sequence analysis we addressed the question why lemur monkeys as prosimians represent such excellent hosts for oral BSE infection. For the preparation of genomic DNA we used peripheral blood leukocytes of a white ruffed lemur (*Lemur/Varecia variegatus variegatus*) and of a black lemur monkey (*Lemur macaco albifrons*; materials courtesly provided by the Zoological Gardens of San Francisco, USA). Using the degenerated primers FWA and FWH (Wopfner *et al.*, 1999) we generated PCR products corresponding to amino acid residues 27–192 (human numbering system; GenBank accession number AF177293). From agarose gel analysis it was evident that the lemurs we have analyzed might be heterozygous, carrying two alleles of different size (Figure 1). We sequenced both alleles directly from PCR products and upon subcloning of individual clones into suitable vectors. A comparison with known PrP sequences demonstrated that the generated PrP sequence was a unique and novel PrP sequence and that we have identified a PrP polymorphism in heterozygous animals. The lemur PrPs we have analyzed encoded methionine at codon 129 and did not encode the linear epitope mapped for the monoclonal anti-PrP antibody 3F4 (MKHM; residues 109–112) (Figure 2). Reactivity with this antibody was shown by Bons and colleagues in immunocytochemistry and immunoblotting
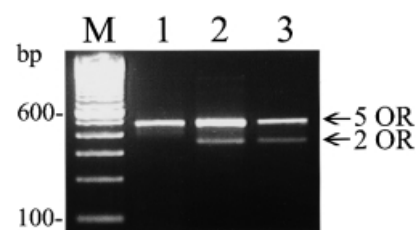
Fig. 1 PCR Analysis of Lemur PrP Genes.
Internal primers encompassing the octarepeat elements were used (LE3: AAC ACT GGG GGG AGC CGA TAC; LE4: CTT GAT GGT GAT ATT GAC GC). In lane M a DNA size marker is shown (100 bp ladder). Lane 1 represents DNA amplified by PCR from human genomic DNA, lane 2 contains PCR products from genomic DNA of a black lemur, and lane 3 of a ruffed lemur. DNAs in lane 2 and 3 represent a PrP polymorphism in heterozygous animals. Depending on the PCR conditions one of the two alleles was more pronounced in intensity.

Fig. 2 Alignment of Octarepeat Sequences of Various PrPs.

The deduced PrP amino acid sequences from residue 51 to 119 were aligned (numbering system related to human PrP; Schätzl *et al.*, 1995; Wopfner *et al.*, 1999). The lemur monkey was compared to cattle, sheep, goat, marmoset monkey (representing new-world monkeys), macaque (representing old-world monkeys), chimpanzee (representing great apes), and human. The octarepeat structure is indicated by stretches of 8 (9) amino acids interrupted by spaces. The number of octarepeats for a given PrP allele is indicated in brackets. Mammalian PrPs usually consist of 5 octarepeats; the bovine PrP can be heterozygous, having either 5 or 6 octarepeats. For goats the recently described polymorphic allele encoding 3 octarepeats is shown (Goldmann *et al.*, 1998). The first two lines depict the polymorphism in lemurs with a short allele encoding two and a long allele encoding five octarepeats.

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                51       60                          75           90          105
lemur (2)*:     PQGGG-WGQ -------- -------- -------- -------- PHGGG-WGQ GGGSHGQWNKPSKPKTNMKHVAGAAAAG

lemur (5):      PQGGG-WGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGG-WGQ GGGSHGQWNKPSKPKTNMKHVAGAAAAG

cattle (5/6):PQGGGGWGQ PHGGGWGQ PHGGGWGQ PHGGGWGQ PHGGGWGQ PHGGGGWGQ GG-THGQWNKPSKPKTNMKHVAGAAAAG

sheep (5):      PQGGGGWGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGGGWGQ GG-SHSQWNKPSKPKTNMKHVAGAAAAG

goat(3):        PQGGGGWGQ -------- -------- -------- PHGGGWGQ PHGGGGWGQ GG-SHSQGNKPSKPKTNMKHVAGAAAAG

marmoset (5):PQGGG-WGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGG-WGQ GGGTHSQWNKPSKPKTNMKHVAGAAAAG

macaque(5):     PQGGGGWGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGG-WGQ GGGTHNQWHKPSKPKTSMKHMAGAAAAG

chimp (5):      PQGGGGWGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGG-WGQ GGGTHSQWNKPSKPKTNMKHMAGAAAAG

human (5):      PQGGGGWGQ PHGGGWGQ PHGGGWGQ -------- PHGGGWGQ PHGGG-WGQ GGGTHSQWNKPSKPKTNMKHMAGAAAAG
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*: Number of octarepeats for a given allele in brackets; polymorphic alleles of lemur (2/5), goat (3/5) and cattle (5/6) are shown.

experiments (Bons *et al.*, 1999). Whether this might be explained by interspecies variability within various lemur subspecies has to be determined.

Mammalian PrPs have a characteristic repeat structure located at the N-terminal part, usually consisting of a five time repetition of 8 (9) residues (octarepeat sequence PHGGGWGQ). Avian PrPs consist of 6 – 9 repeats of 6 residues (Wopfner *et al.*, 1999), and a marsupial PrP consisting of 4 repeats of 9 – 10 residues was also described (Windl *et al.*, 1995). Interestingly, the short lemur PrP allele showed the deletion of three entire octarepeats, yielding the shortest known PrP with only two octarepeats (Figure 2). The functional significance of the repeat structure is not entirely clear, but evidence is growing in support of a functional role of this element in copper binding (Hornshaw *et al.*, 1995; Brown *et al.*, 1997; Viles *et al.*, 1999). Some minor inter- and intra-species variability within the repeat structure is known (Wopfner *et al.*, 1999), and recently a goat PrP polymorphism was described with three octarepeats in one allele (Goldmann *et al.*, 1998). Apparently, goats encoding three octarepeats showed an improved resistance against infection with caprine prions (Goldmann *et al.*, 1998). Loss of one allele has no effect on prion disease susceptibility in humans and primates (Schätzl *et al.*, 1995). On the other hand, the insertion of two and more octarepeats in human beings is clearly associated with familial Creutzfeldt-Jakob disease (Owen *et al.*, 1989; Palmer and Collinge, 1993). Interestingly, the presence of the N-terminal part of PrP (*i.e.* residues 23 – 90) is dispensable for prion protein infectivity (Rogers *et al.*, 1993; Fischer *et al.*, 1996), although recent data suggest a role in modulating prion disease pathogenesis (Weissmann and Flechsig, personal communication).

Our finding that lemurs encode a truncated PrP allele raises several important questions. First, is the octarepeat structure, consisting of two octarepeats, still functional in copper binding? Recent data described the cooperative nature of PrP-copper complex formation and suggested a functional requirement for four octarepeats (Viles *et al.*, 1999). In line with this one would predict that the lemur PrP with two octarepeats is significantly impaired in its biological function. A second aspect is whether this truncation is related to the remarkable oral infectibility of lemurs with BSE-derived prions. And finally, one could argue that this genotype alone might favour development of a prion disease, even in the absence of exogenous infection. Future animal studies are certainly needed to decipher the putative correlation between the PrP genotype described here and the outstanding oral susceptibility of lemurs to BSE-derived prions.

Acknowledgements

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